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NUTRITION REVIEWS

VOL. 12 SEPTEMBER 1954 No. 9

EARLY EXPERIENCES WITH PYRIDOXINE—A RETROSPECT

The key to the isolation of pyridoxine was the sensitivity of the method used, making possible approximate pyridoxine determinations in our fractions in less than twenty-four hours. The method itself was made possible by the discovery that the third member of the vitamin B-complex (vitamin B_6) was multiple in nature and that besides vitamin B_6 (pyridoxine) at least one additional vitamin was required by the rat. It was the addition to the basal diet of concentrates containing this new vitamin (pantothenic acid) that gave the method its sensitivity. Dr. Juke's cooperation greatly facilitated this discovery which largely escaped the attention of most university research workers. Its importance was, however, recognized and fully appreciated by the research workers at Merck and Company and those at Mead Johnson and Company. Merck and Company implemented their recognition of this work with an unsolicited research grant of a substantial sum of money.

The animal work was carried out in a small room measuring approximately 9 x 11½ feet. It had one large rack of cages that housed about 200 rats. Visitors could scarcely believe that pyridoxine was isolated with such limited animal facilities. The work was made possible by the rapid turnover of our experimental animals. If rats were fed fractions in the morning we could frequently tell that same evening which one carried the bulk of the activity. Often the active fraction was subjected to further fractionation on the same evening and new fractions were prepared and ready for feeding the following morning. The work day seldom ended before 2:00 a.m., and Saturdays and Sundays were generally work days. It was thus largely by hard work and speedy assays that pyridoxine was isolated.

It took, however, a lot more than hard work to do the job. The cooperation of Mrs. Myrtise Krause in the animal room and of about 3 WPA workers was of the greatest importance. Also of decisive importance was the use of plant equipment at the Vitab Company, made available to us through the courtesy of Mr. Edward H. Heller and Mr. A. K. Humphries. When Nopco Chemicals purchased the Vitab Company Mr. Perc Brown and Mr. Ralph Wechsler, both vice presidents, called on me personally and assured me that the plant equipment would continue to be at my disposal. I am very grateful to these men for their generous cooperation.

I watched the race for the isolation of pyridoxine as a spectator and had no thought of being a participant. It didn't occur to me that there was the remotest chance for anyone with such limited facilities as ours to participate in a race against such giants as Merck and Company, Kuhn and his collaborators at Heidelberg in Germany, György and his co-workers, and the group of nutritional investigators at the Lister Institute in England.

In one of my acetone-containing fractions some 5 to 10 mg. of crystals had separated out. I was sure that this crystalline material was an inactive salt of one kind or another since previous experience had shown that I was particularly adept at isolating inactive crystalline material. I poured off the mother liquor and gave the crystals a wash, mainly to recover the vitamin-containing liquors. Since I was sure the crystals were inactive I dissolved the whole of them in a bit of water and gave it to Mrs. Krause to test with rats. The next day she came to me with a puzzled look and asked, "What did you put in that water?" She then

showed me the rats that had consumed the "water." Their appetites had been so stimulated and they had eaten so much food during the night that they almost took the shape of spheres. These rats swallowed my first batch of crystals in one gulp.

It now occurred to me that the isolation of pyridoxine was possible even by workers with limited means. Another batch of crystals was then prepared. It took over a month, as the procedure was laborious and time-consuming. This time I had about 120 mg. of crystals. They were repeatedly tested and always found active, and yet I experienced much difficulty in believing that these crystals were, indeed, the elusive vitamin.

During this period the building, a veritable firetrap, caught fire at about 2:00 a.m. The telephone connections were burned out, so I had to run to the foreman's house to call the fire department. When the firemen came, I suddenly remembered my crystals. My second batch of crystals was in that burning building! Fortunately, the fire was put out and my crystals were saved. There were, however, over 200 roasted chickens. We now have a sprinkler system in the building, a facility impossible of attainment before the fire. The Poultry Industry of California rewarded me with a leather wallet for saving the Poultry Building.

The day after the fire, I discovered that I had suddenly become famous. I made the front-page headlines of many of the leading newspapers in this area. In one of them I was depicted as the absent-minded professor so completely absorbed in his work that he did not know the building was on fire. At the sound of the noises of the fire-fighting equipment outside, I allegedly poked my head out of the window and, completely oblivious of the fire, asked what the noise was all about. The reporters just didn't believe I was in the laboratory doing research at 2:00 a.m. They had little understanding of the "laboratory rats" who are responsible for so much of our research.

When I was finally convinced that I had, indeed, crystallized pyridoxine—I called it Factor 1—I sent a note to *Science* announcing its isolation. The note appeared in February 1938. The next month, March 1938, the *Proceedings of the Society for Experimental Biology and Medicine* appeared with the paper by Keresztesy *et al.*, also announcing the isolation of pyridoxine. The journal, which appeared in March 1938, carried the date of February 1938.

I was richly rewarded for all my efforts. It came in the form of two letters: one, a letter of congratulation from my revered teacher, Professor E. B. Hart, and the other from the Lister Institute in England under the signature of T. F. Macrae and it read in part, "We have a great respect for your work and it is some conolation to us, since we have failed, that you have won the vitamin B_6 isolation race."

Shortly afterward Dr. Randolph T. Major, director of research for Merck and Company, paid me a visit. He wanted to know if I was going to patent the process for the isolation of pyridoxine. I told him I wasn't interested in patents. He then asked if I would disclose the process to him. "Certainly," I answered. "When?" he asked. "Now," was my answer, and I began to write out the procedure. "Now, wait a minute," he said. "Let me first get permission for this from your dean." Dean Hutchison gave his permission and Dr. Major got the procedure. It is this kind of consideration and thoughtfulness that makes Dr. Major such a fine human being as well as a great director of research.

Dr. Major and I discussed the future of the vitamins from an industrial point of view. I ventured the opinion that vitamins in the form of natural concentrates had the greatest future. Dr. Major insisted that it was the synthetic vitamins that had the greatest industrial potential and he steered Merck and Company into a vast developmental program geared to the production of synthetic vitamins. Each vitamin had first

to be isolated from natural materials, its structure determined and a method for its synthesis worked out before it could be produced. The success that has attended this program has been phenomenal. Thanks to this courageous decision by Dr. Major, many pure vitamins were made increasingly available for nutritional investigations and they have had a tremendous impact upon the character and quality of research in nutrition.

Some time later I had a visit with Dr. A. R. Todd who took part in the chemical work on the isolation of pyridoxine at the Lister Institute in England. We compared notes on the procedures that each one of us used in our work and they were surprisingly similar. There seemed to be but one major point of difference—I had recrystallized the phosphotungstates and he didn't. That one additional step apparently made the difference between success and failure. His only remark was, "Now, why didn't I think of that?"

At about this time we began our long-distance cooperative research program with Dr. Paul Fouts and Dr. Oscar Helmer at the Eli Lilly Research Laboratory at the Indianapolis City Hospital. We, at California, were to subject our vitamin B-complex concentrates to exhaustive study with rats and chicks and then they would go to Drs. Fouts and Helmer at Indianapolis for further study with blacktongue dogs and human pellagrins. The objective of the cooperative study was to secure comparative information on the vitamin B-complex with all the animals mentioned. The big prize was the pellagra-preventive factor.

One of the chief results of this cooperative effort was the discovery by Drs. Fouts and Helmer that pyridoxine-deficient dogs develop a microcytic hypochromic anemia curable with crystalline pyridoxine. They also first described pantothenic acid deficiency in dogs.

While these studies were in progress, I visited the late Dr. J. C. Drummond in London where we discussed the possible role of nicotinic acid in nutrition. He was convinced and he convinced me that nicotinic acid played an important role somewhere in the then confused vitamin B-complex picture. I wrote my laboratory to secure some nicotinic acid and on my return it was subjected to exhaustive study with uniformly negative results. Thus, I had nicotinic acid in California and there were available dogs with blacktongue and human pellagrins at the Indianapolis City Hospital. It would seem almost impossible, under these circumstances, somehow or other not to discover the pellagra-preventive vitamin. I surmounted this impossibility by merely neglecting to wrap up a bit of nicotinic acid in some paper and address it to Drs. Fouts and Helmer at the Indianapolis City Hospital. I did just that about a year later after the Wisconsin group (Elvehjem, Madden, Strong, and Woolley) showed that nicotinic acid cured blacktongue in dogs, so that Drs. Fouts and Helmer were among the first, if not the first, to feed nicotinic acid to pellagrins. Lady Luck is very selective in the way she passes out her favors.

Our next problem was directed toward the determination of how pyridoxine functioned in the animal and this study led to the isolation of xanthurenic acid. This was made possible because we were short of research funds. The study required the use of metabolism cages so we could collect feces and urine. We had no money to buy cages constructed out of monel metal or stainless steel. In fact, we had no money to buy any kind of metabolism cage, so one of our WPA men built a metabolism cage out of a pie plate, some sheet metal and hardware cloth. The rat urine corroded the metal and it became rusty. Urine voided by the rats came in contact with the iron salts of the corroded metal. When pyridoxine deficiency appeared in the rats the urine that we collected from them had a green color while the urine from the pyridoxine-fed rats retained its normal color even though it also

made contact with the corroded metal of the cage. Urine obtained directly from the bladders of the pyridoxine-deficient rats had the normal yellow color of rat's urine which turned green upon the addition of an iron salt. We then realized that the urine from pyridoxine-deficient rats contained an unidentified metabolite which was absent from the urine of the pyridoxine-fed rats. This unknown metabolite evidently reacted with an iron salt of the corroded cage to form a green iron derivative. Had we been able to afford monel metal or stainless steel cages, we would have missed xanthurenic acid.

When the green urine was filtered free of contaminated rat's hair, feces and spilled food, I noticed that the filter paper was a shade darker than might be anticipated from the color of the urine. This suggested a method for the purification of this green pigment-producing metabolite. Filter paper was pulped by vigorous shaking in water and poured into a tube, making a chromatographic column with paper packing. An iron salt was added to the urine to convert it to a green pigment and it chromatographed quite well. The iron-complex of the unknown metabolite would not crystallize so we started all over, working with the metabolite itself. When the urine was saturated with NaCl, a broad yellow band separated out on the paper chromatograph. With careful manipulation, the urinary constituents could be washed through the column before the yellow compound came through. The yellow compound turned green when iron salts were added. We knew, therefore, that our unknown metabolite was yellow in color. We could thus follow it visually and check it by the addition of iron salts. These observations greatly facilitated its concentration and ultimate isolation.

The problem now was to get rid of the NaCl. The yellow material was concentrated in vacuum and as the slightly discolored NaCl separated out it was discarded. During the procedure we kept losing the yellow metabolite, indicating that we were working with a very unstable compound. It was discouraging to prepare concentrates of the yellow metabolite only to lose it. After altogether too long a time I took some slightly discolored salt that I had been discarding and examined it under the microscope. Among the crystals of NaCl were some beautiful yellow crystals. The slight "discoloration" was in fact the eagerly sought-for metabolite. A sufficient amount of crystalline material was soon obtained. Dr. Haagen-Smit and his collaborator Dr. Roboz at the California Institute of Technology quickly determined the structure of the unknown metabolite and identified it as xanthurenic acid. They suggested that it might be a metabolite of tryptophan metabolism. It was quickly established with rats that xanthurenic acid was an aberrant metabolite of tryptophan metabolism in pyridoxine-deficient rats.

My experience with xanthurenic acid convinced me that the day of "push-button" research has not yet come. The investigator has to do more than sit at his desk, outline experiments and examine data.

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NUTRITION AND THE COURSE AND OUTCOME OF PREGNANCY

The effect of diet upon the course and outcome of pregnancy has engaged the efforts of many investigators. Although an adequate diet during pregnancy is conceded generally to be of importance to the health both of the mother and the infant, there is lack of agreement concerning how inadequate diets may influence the course of

pregnancy. In the study of this relationship a number of approaches have been utilized. J. H. Ebbs, F. F. Tisdall, and W. A. Scott (*J. Nutrition* **22,** *515 (1941)*; *Nutrition Reviews* **1,** *276 (1943)*) gave food supplements to a group of 90 pregnant women in the last half of pregnancy. These women had been found to consume relatively poor diets as determined by diet records. The course and results of pregnancy in this group were compared with those in a second group of 120 women who remained on poor diets without supplementation and with a third group of 170 women whose diets during pregnancy were described as adequate or good. The rates of occurrence of abortions, premature births, stillbirths and neonatal deaths were significantly higher in the group of women on the poor but unsupplemented diet. In addition, the women who consumed either the supplemented or the good diet proved to be better obstetric risks, with lower incidence of toxemia and fewer difficulties during labor, delivery and the postpartum period. M. I. Balfour (*Lancet* **I,** *208 (1944)*) reported the results of a study of nearly 20,000 pregnant women from lowest income groups in England and Wales. It was determined that important advantages for the infant resulted when diet supplements of yeast, vitamins A and D, as well as of calcium, phosphorus and iron were afforded. Stillbirth and neonatal death rates were significantly lower in the group of women given these diet supplements than in a control group without supplements. No difference in maternal death rates was noted and although the incidence of toxemia was less in the supplemented group, the difference did not prove to be significant by statistical tests.

Another approach has been to evaluate the diet histories of women at intervals throughout the course of pregnancy. In a carefully undertaken study of this type, B. S. Burke, V. A. Beal, S. B. Kirkwood, and H. C. Stuart (*Am. J. Obstet. Gynec.* **46,** *38 (1943)*; *Nutrition Reviews* **1,** *386 (1943)*) found important relationships between the diet during pregnancy and the condition of the infant at birth. Stillbirths, neonatal deaths and premature births occurred much more frequently in the group of women on poor diets than in those on good diets. The infants born to mothers on poor diets were of smaller average weight and shorter length at birth. In addition, in this group were the majority of infants with major congenital defects and those described as "functionally immature" at birth. Less pronounced indications were found of an effect of poor diet on the health of the mother during pregnancy. However, there was a significant relationship between poor diet and the occurrence of toxemia.

A subsequent publication by B. S. Burke, S. S. Stevenson, J. Worcester, and H. C. Stuart (*J. Nutrition* **38,** *453 (1949)*), where the dietary material is divided into four groups, shows clearly the consistent trend toward lower physical ratings of infants with lower maternal dietary ratings and particularly the abrupt increase in the percentage of stillbirths, neonatal deaths, premature infants and those with major congenital defects when the maternal dietary rating reached its lowest level; this is an extremely deficient diet even in the minds of the most conservative. This paper also makes clear that mothers of the 216 infants previously discussed (*Am. J. Obstet. Gynec., loc. cit.*) changed their food habits little in subsequent pregnancies and it is likely that the dietary ratings given represent not only diets of these women during pregnancy but in the majority of cases their long-time food habits. Hence, any association found between prenatal dietary rating and condition of infant at birth may be due either directly or indirectly to the diet during pregnancy, or to long-established food habits which tend to control a woman's nutritional state at the time she enters pregnancy, or to some combination of these two factors. It is possible that nutrition during the latter part of pregnancy is a factor in certain relation-

ships shown to exist, while long-established food habits may be important to both mother and fetus in other ways.

The effect of severe food restriction in large population groups upon reproductive capacity has been evaluated by a number of authors. C. A. Smith (*J. Pediat.* **30,** *229 (1947)*) studied the effects of semistarvation in war-torn Holland in 1945. At this time there were manifestations of severe general malnutrition in urban populations. About 50 per cent of the women became amenorrheic and were presumed to be infertile. The birth rate was reduced by about two-thirds. The average weight and length of infants at birth were significantly reduced. Rates of occurrence of premature births and of congenital malformations were increased but not to significant degrees. However, there was no increase in stillbirths or neonatal deaths associated with the period of severe food restriction.

Although not all of the observations made in the study of the relationship between the quantity and quality of the diet and the outcome of pregnancy are consistent with all others, the conclusion has seemed warranted that poor diet of the mother during the period of pregnancy may compromise her health as well as that of the infant. A further study of this relationship was undertaken in 1945 at the Vanderbilt University School of Medicine. This investigation was an ambitious one in which six different laboratories and clinical services were involved. It proposed an evaluation of the state of nutrition of each woman who presented herself for prenatal care and delivery at the Vanderbilt University Hospital during a three-and-one-half-year period. It was anticipated that such a group of women would be representative of the total population of pregnant women. The only factors of original selection were those which contributed to attendance at this, a university clinic, serving a medically indigent population group. A total of 2338 women participated in the study. Of this number 292 have been excluded in the analysis of data because of incomplete obstetric records or for similar causes.

The results of this investigation have been published in a series of four papers. The first paper describes the organization of the study, the methods used and the characteristics of the group of women who participated (W. J. Darby *et al.*, *J. Nutrition* **51,** *539 (1953)*). Some of the considerations which were important to the initiation of the investigation are stated. It had been noted earlier that in the experience of the obstetric service at the Vanderbilt University Hospital the incidence of puerperal fever and of toxemia was greater in ward patients than in patients on the private service, whose incomes were greater and whose diets were presumably more adequate. In addition, a number of nutrition surveys of the general population had been undertaken in the local area. It had been observed that the dietary pattern of pregnant and lactating women was similar to that of women who were not in the gravid state. Moreover, it seemed that these women did not increase food intakes during pregnancy, an event which might lead to nutritional deficit because of the enhanced nutrient requirements of this period. Laboratory studies done in these surveys had demonstrated that pregnant women frequently had low levels of hemoglobin, serum protein, and vitamin A and ascorbic acid in serum. This fact deepened the suspicion that the gestational period is often associated with a compromised state of nutrition of the mother.

The investigation was planned so that information might be afforded concerning the changes in nutritional physiology during pregnancy and the postpartum period. It was hoped also that the study would allow for a critical evaluation of the role of nutrition as a factor determining the healthfulness of pregnancy.

The data compiled on each woman included the usual medical and obstetric history and examination; special nutritional

examination; determination of blood hemoglobin, red blood cell count, packed cell volume, serum ascorbic acid, serum carotene and vitamin A, total serum protein and serum albumin; results of urinary load tests for thiamine, riboflavin and N′-methylniacinamide; and a seven-day diet record. This information was obtained when the patient first presented herself for prenatal care. Once during each subsequent trimester the diet records, physical examination and laboratory tests were repeated. In addition, physical examinations and laboratory determinations were done six weeks after delivery of each subject.

The dietary management of these patients was unchanged from the usual practice in that clinic. The obstetrician counselled each patient concerning the advisability of drinking milk and of obtaining added food sources of iron. Some patients were given nutrient supplements as indicated. Actually 27 per cent of the group of women received a supplement of one type or another. Obese patients were asked to reduce intakes of sugars and starches.

The medical records were abstracted for all pertinent information concerning the course of pregnancy in the mother and the physical state of the infant at birth. The characteristics of the group of pregnant women and the results of pregnancy are described in another paper (W. J. McGanity *et al.*, *Am. J. Obstet. Gynec.* **67,** *491 (1954)*). Of the 2046 patients included in the group, 32 per cent were observed during the first pregnancy. Seventy-nine per cent of the group were less than 30 years of age. All women came from homes in the low to moderate income range. Pre-existing disease which might have influenced the ultimate fetal or maternal outcome of pregnancy was present in 225 women, or 11 per cent.

Complications of pregnancy of most common occurrence were anemia (hemoglobin concentration less than 10 g. per cent) in 232 women, pre-eclampsia in 93, eclampsia in 10, premature separation of the placenta in 24 and placenta previa in 7.

There were 2068 infants and fetuses born as a result of the 2046 pregnancies studied. Ninety-two per cent of these were mature infants, weighing more than 2500 g. at birth. Premature or immature infants (400 to 2499 g.) accounted for about 7 per cent. There were 20 abortions and 30 stillbirths. Of the 2018 live births, 42 died in the neonatal period.

There were complications in delivery of 7.6 per cent of the women and cesarean section was required in 65 patients, or 3.2 per cent. No maternal deaths were recorded, but postpartum infection occurred in 110 cases and hemorrhage in 21.

The results of evaluations attempting to relate nutritional status to obstetric performance in this group of patients are discussed in a paper by W. J. McGanity and his associates (*Am. J. Obstet. Gynec.* **67,** *501 (1954)*). It is emphasized that the procedures for determination of the state of nutrition were applied as each pregnancy progressed and that these represented nutriture prior to the development of fetal and obstetric abnormalities. There were 25 abnormal conditions of gestation which were studied. The occurrence of each of these was investigated to ascertain possible differences in nutritional status that may have existed between patients developing these complications and others without abnormalities of pregnancy.

The diet records of the entire group of 2046 women indicated average daily nutrient intakes somewhat below those recommended for women during pregnancy in the Recommended Dietary Allowances of the National Research Council. Moreover, nutrient intakes tended to decrease in the third trimester, making the deficit greater, since recommendations are for increased food consumption in the latter half of pregnancy. The average daily intake of calories was 2130 during the first trimester, 2200 during the second trimester and 2020 calories in the

third trimester. There was considerable variation about average values and many individuals consumed much less food than the average and others much more. Physical manifestations of frank deficiency disease were entirely absent, while subclinical signs were noted, with variations in terms of examiners, more frequent occurrence late in pregnancy, increased incidence in older women and after several pregnancies, and with seasonal variations. The results of laboratory determinations are fully discussed in a report by W. J. Darby *et al.* (*J. Nutrition* **51,** *565 (1953)*).

Toxemia of pregnancy was recorded as occurring in 103 patients. Ninety-three of these were cases of pre-eclampsia and 10 were eclampsia. The average nutrient intake of these women tended to be lower than in the study group as a whole throughout the prenatal period, and was significantly lower in the first and third trimesters. The chief deficits in the first trimester were in protein, iron, riboflavin and niacin, while in the third trimester there were significant decreases in intake in calories and niacin. There was a higher proportion of obesity in the women who developed pre-eclampsia than in the total group. The authors feel that since 35 per cent of the women in the group were obese and were more likely to have received advice from their physicians to limit their nutrient intake, the lower level of food consumption is a result rather than a cause of toxemia. The laboratory data indicated an elevation of levels of serum total protein and albumin in the second trimester. In addition, the urinary excretion of N′-methylniacinamide was significantly increased in the puerperal period of the women who developed toxemias.

There were 265 patients with puerperal complications. This group differed from the total study group in no significant way in terms of nutrient intake. In addition, the results of physical examinations failed to indicate an increased incidence of subclinical signs of deficiency. However, it was determined that although intakes of iron and vitamin A were adequate throughout pregnancy, hematologic and serum vitamin A values were significantly lowered. The authors suggest that these lower values may reflect increased susceptibility to infection during the puerperal period.

Seventy-two stillbirths or neonatal deaths occurred in the offspring of 71 mothers. There were no significant deviations in diet pattern of these women from the total study group. Serum total protein and albumin tended to be elevated throughout the prenatal period, however. This finding was not the result of inclusion in the group of cases of toxemia, since fetal loss occurred because of toxemia in but 11 per cent of the group. Obesity occurred twice as frequently in this group as in all of the patients.

One hundred and twenty-three infants were born prematurely. The only deviation in nutrient intake of this group of mothers was in ascorbic acid. More than the expected number consumed less than 20 mg. of this vitamin per day in the second and third trimesters. Giving added significance to this finding, serum levels of ascorbic acid were decreased in the latter two trimesters. Although there was a high frequency of manifestations of undernutrition in this group, specific signs of ascorbic acid deficiency were not importantly increased. Therefore, the authors are not of the opinion that a low level of nutrition relating to ascorbic acid directly conditioned the occurrence of premature births.

No clear relationship was demonstrated between the nutritional status of these women and the occurrence of intractable nausea and vomiting of pregnancy, excessive blood loss at delivery, inability to nurse infants, abortions, and a number of other obstetric abnormalities.

Finally, another type of analysis was undertaken. This was to relate the occurrence of abnormalities in the course and outcome of pregnancy to major deviations in nutrient intake. Although the results of

this analysis are not given in detail and it is stated that they will be published elsewhere, two very important observations are recorded. The first states that there was no decrease and no increase in obstetric or fetal complications in the women whose intake of nutrients exceeded the mean of the entire group by one or two standard deviations. The second indicates that there was a significant increase in incidence of pregnancy disease in general and in preeclampsia and eclampsia in particular when dietary intakes fell approximately one standard deviation or more below the mean of the total group. The most important correlations were in reduction of caloric intake to less than 1500 calories and of protein to less than 50 g. per day. The statement is made that abnormalities of pregnancy seemed to be the cause of the reduced nutrient intake rather than the result of it. It is not totally clear on what facts this conclusion is based.

These reports contain much information relative to the dietary habits, nutritional status and obstetric performance of a large group of women representative of a low- to middle-class social group. Diets were grossly deficient in but a few, and no relationship to the development of abnormalities of pregnancy is strikingly demonstrated. These findings must be added to those reported earlier in the study of this important relationship. They do not negate or provide evidence in opposition to certain of the earlier observations demonstrating more positively a relationship between poor diet and hazards to maternal and fetal health. Of greatest importance is the fact that they record in detail the changes which occurred in nutritional physiology during the course of pregnancy in this relatively large group of women.

THYROID-ADRENOCORTICAL INTERRELATIONS

When malnutrition develops after serious injury or operation considerable complications ensue—wound healing is delayed, morbidity is prolonged and mortality is increased. Numerous investigators have reported changes in protein, fat, carbohydrate, vitamin, water and electrolyte metabolism after injury, especially in the early post-trauma period. Protein metabolism, in particular, has been studied, and it has been observed that characteristically an injured individual excretes excessive amounts of nonprotein nitrogen for two to seven weeks following injury. During this time, he is in negative nitrogen balance and is gradually losing body protein.

As pointed out previously (*Nutrition Reviews* **11,** *265 (1953)*), the pathogenesis of the increased urinary excretion following injury has not yet been established. Among the factors which have been considered in this regard are (1) autolysis and absorption of tissue breakdown products, (2) inactivity, (3) specific amino acid deficiency, (4) renal dysfunction, (5) increased proteolytic enzyme activity, (6) reduced dietary intake, (7) increased metabolic rate, and (8) imbalance of endocrine activity (*Ibid.* **3,** *40, 86, 253 (1945)*; **4,** *6 (1946)*; **5,** *46 (1947)*; **6,** *20, 161 (1948)*; **7,** *123 (1949)*; **8,** *137 (1950)*; **9,** *257 (1951)*; **10,** *99, 195 (1952)*; **11,** *265 (1953)*). It is to this last possibility, an imbalance of endocrine activity, that I. S. Goldenberg, L. Lutwak, P. J. Rosenbaum, and M. A. Hayes (*Surg. Gynec. Obstet.* **98,** *513 (1954)*) have recently directed their attention.

Possible thyroid-adrenocortical interrelationships following operation were investigated in 5 patients. The patients studied included a hypothyroid female, 2 euthyroid females, a slightly hyperthyroid 55 year old male, and a slightly hyperthyroid female who had been given propylthiouracil for three weeks prior to operation. In this last patient, propylthiouracil was continued in

the postoperative period of study. In 4 of the 5 patients the operative procedures were relatively minor; in one patient a total hysterectomy and bilateral salpingectomy were performed. All operations were carried out under general anesthesia. The patients were studied for one week preoperatively and one week postoperatively. During the preoperative control period each patient ate a diet providing 1600 calories and 10 g. of nitrogen per 1.73 square meters of body surface. No mention is made of the proportions of calories supplied by fat and carbohydrate. Dietary intake was low, and not reported, on the day of operation. Beginning on the first postoperative day, 4 of the patients ate at the preoperative control levels, while the fifth received only saline, glucose and water parenterally.

Daily urinary nitrogen, sodium, potassium, total inorganic phosphorus, uric acid, creatine, creatinine, 17-ketosteroids, corticosteroids and Pettenkofer chromogens were measured. Fecal sodium, nitrogen, and potassium were determined in three- to four-day periods. Uptake by the thyroid of radioactive iodine was measured one, three, six and twenty-four hours, and then daily, following its administration, orally or intravenously. Urinary excretion of the radioactive iodine was measured at twelve and twenty-four hours, and then daily, following administration. The serum level of butanol-extractable iodine (which corresponds to the protein-bound iodine) was measured at intervals of one or more days.

The authors state that increased thyroid activity occurred immediately after operation and was associated with an increase in nitrogen excretion. Increased adrenocortical activity then followed and initiated readjustments which counteracted the thyroid effects, and resulted in a return of nitrogen excretion towards normal. They interpret their findings as indicating that the increased urinary nitrogen excretion after operation is consequent to disequilibrium between the activity of the thyroid and the adrenal cortex.

The authors may be right in their concept, but it would seem that their reported data do not allow so clear-cut a conclusion. Summaries of 2 of the 5 patients reported are as follows:

The first patient was a 54 year old woman who had undergone a total thyroidectomy one year previously for a nonfunctioning thyroid cancer. She was admitted because of the presence of a mass in the operative scar. She had been maintained on thyroid, but this was omitted three weeks before study. A low uptake of radioactive iodine by the thyroid, low urinary creatine excretion, and low serum butanol-extractable iodine concentration suggested that she was mildly hypothyroid at the time of operation.

Operation consisted of excision of the mass. No recurrent carcinoma or functioning thyroid tissue was present in the mass which revealed granulation tissue and foreign body reaction.

Radioactive iodine uptake twenty-four hours postoperatively was higher than preoperatively one and three hours after administration of the test dose, but thereafter no difference was apparent. The twelve-hour urinary excretion of radioactive iodine was less postoperatively, but similar cumulative amounts were excreted thereafter. The concentration of serum butanol-extractable iodine did not change, while there was a slight increase in urinary creatine excretion (from about 0.1 mg. to 0.3 mg. on the third postoperative day). There was an increase in urinary corticosteroid excretion on the fifth postoperative day (no subsequent observations reported), decrease in the Pettenkofer chromogens and slight decrease in the 17-ketosteroid excretions. The uric acid:creatinine ratio did not change. Sodium was retained transiently (one day); potassium and urinary inorganic phosphorus excretions were unchanged. Nitrogen balance was slightly positive preoperatively, and became somewhat more

positive postoperatively. In summarizing these data the authors state, "With evidence of increased thyroid activity, shown by an increase in creatine excretion, and evidence of increased adrenocortical activity, shown by an increase in corticosteroid excretion, there is a definite increase in nitrogen retention, a finding not to be predicted from activation of either system independently." It may be questioned whether the reported data unequivocally support this conclusion.

The fifth patient studied was an 18 year old girl with a large tibial spur. To study the metabolic responses to operation in the presence of a thyroid gland whose activity was suppressed by propylthiouracil, she was given 600 mg. of this drug daily for eleven days. At the end of this time, the radioactive iodine uptake by her thyroid twenty-four hours after administration was 14 per cent of the administered dose. Butanol-extractable iodine concentration in the serum was normal. The dose of propylthiouracil was then increased to 800 mg. daily and continued at this level for the five remaining preoperative days and during the postoperative period. Operation consisted of the excision of a benign tibial bone spur.

Uptake by the thyroid of a tracer dose of radioactive iodine given one hour postoperatively was similar to the preoperative observations during the first twenty-four hours following administration, but at forty-eight hours the postoperative uptake was 20 per cent as compared with 14 per cent at a comparable time preoperatively. The concentration of serum butanol-extractable iodine did not change, but urinary creatine excretion increased. The patient was in slight negative nitrogen balance during the preoperative period. There was an increase in urinary nitrogen excretion during the first three postoperative days with a return towards the preoperative level on the fourth and fifth days.

The authors state that the increased nitrogen excretion in this patient occurred coincidentally with the increased postoperative thyroid activity and a delayed adrenocortical response, and that nitrogen excretion decreased as adrenocortical activity became prominent. However, the data in regard to adrenal activity presented do not give so definite a picture of the activity of the adrenal cortex. For example, there was no urinary excretion of corticosteroids in the postoperative period greater than that seen in the preoperative period, and the excretion was as high on the first postoperative day as on any other day. Urinary excretion of the 17-ketosteroids was fairly consistent during the postoperative period, and the highest daily excretion on the third postoperative day was no higher than that seen on one of the preoperative days. Excretion of the Pettenkofer chromogens was higher on the first and third postoperative days than during the preoperative period. but about the same as preoperatively on the fourth and fifth postoperative days. There was little change in the uric acid:creatinine ratio and no potassium diuresis. There was some retention of sodium on the third and fourth postoperative days.

The serum levels of butanol-extractable iodine did not change significantly in any of the patients studied despite changes in thyroid activity as judged by radioactive iodine uptake by the thyroid and urinary creatine excretion. This suggests to the authors an increased turnover of the thyroid hormone with changes in peripheral utilization rate.

M. de G. Gribble and R. A. Peters (*Quart. J. Exp. Physiol.* **36,** *119 (1951)*) have previously shown that urinary nitrogen excretion after injury is less in thyroidectomized rats. These findings were contrary to those of E. A. Sellers, S. S. You, and R. W. You (*Endocrinology* **47,** *148 (1950)*) in burned rats. O. Cope *et al.* (*Ann. Surg.* **137,** *165 (1953)*; *Nutrition Reviews* **11,** *265 (1953)*) have reported no increase in thyroid activity (as measured by radioactive iodine

uptake and protein-bound iodine concentrations in the serum) in burned patients.

The question may be raised whether the measurements made in the studies of Goldenberg *et al.* reflect accurately adrenocortical activity. It is likely that measurements of the blood levels of certain of the adrenal cortical hormone products would be more nearly indicative of the state of adrenal activity than measurements of urinary excretion of corticosteroids, 17-ketosteroids, and Pettenkofer chromogens. In this regard, some studies have revealed a very rapid increase in the blood levels of certain adrenocortical hormone products after operation and injury.

It should also be pointed out that the subjects chosen for study were not of the types most likely to show large increases in urinary nitrogen excretion postoperatively or after injury. A number of studies have indicated that the intensity and duration of this period of protein depletion depends on several factors, among which are the extent of injury and the state of the individual at the time of injury. It is the well-nourished, young adult male who shows the greatest excretion of nitrogen after injury. Further, the extent of the metabolic disturbance parallels, roughly, the extent of the injury. The operative procedures in 4 of the 5 patients in the reported study were relatively minor. Further, it should be pointed out that the patients were studied for only four to five days postoperatively. Peak urinary nitrogen excretion following serious injury or operation is often not seen until after this time and may last for three to seven weeks after severe injury.

It would appear that although Goldenberg and his associates may be right in their concept that the nitrogen excretion after operation is consequent to disequilibrium between thyroid and adrenal cortical activity and other unknown factors, further evidence is required to establish this concept on a firm basis.

EXCESSIVE INTAKE OF VITAMIN A

For a number of years, vitamin A in concentrated form has been readily available to the public. It is widely used in the treatment of skin, renal, gynecologic and ear disorders and, in addition to its "legitimate" uses, it is taken without prescription by many persons in the belief that such self-treatment will afford protection against colds. In view of the large consumption of vitamin A, it is surprising that only 3 cases of chronic hypervitaminosis A in adults have been reported to date. On the face of it, this would seem to indicate that vitamin A is a relatively benign substance (provided that it is not taken in the form of polar bear liver or administered in excessive amounts to infants). The recent experience of A. Gerber, A. P. Raab, and A. E. Sobel (*Am. J. Med.* **16,** *729 (1954)*) with a case of vitamin A poisoning, however, suggests that hypervitaminosis A in adults may pass unrecognized in a number of instances.

Gerber and his associates have reported an unusual case of a 21 year old woman who was admitted to five hospitals over a period of nine years before her illness finally was diagnosed as vitamin A poisoning. Initially, she complained of headaches, double vision and nausea, and when she was examined at hospital "A" a tentative diagnosis of brain tumor was made.

She signed out of hospital "A" and at hospital "B" it was learned for the first time that she had been taking vitamin A in large amounts for eighteen months to treat a skin disorder from which she had suffered since childhood. Because of continued evidence of increased pressure within the cranium with no associated signs of tumor, a diagnosis of serous meningitis (excessive

secretion of cerebrospinal fluid) was made and an operation was performed on the skull to relieve the pressure. This procedure was followed by only temporary improvement and she continued to have bizarre symptoms and complaints. At a third hospital a diagnosis of chronic encephalitis was made and a tantalum plate was inserted at the site of the earlier decompression.

A month later, she entered a fourth hospital where it was noted that she had sparse eyebrows, pigmentation of the skin of the abdomen, enlargement of the liver and spleen, anemia, and pain and spasm of the muscles in the low back area. Somewhat later she was readmitted to the same hospital for removal of a cast which had been applied to relieve the back pain, and at that time it was believed she had an inflammation of the nervous system and of the roots of the sensory nerves leaving the spine. The skin was uniformly hyperpigmented, dry and scaly. Itching was a serious complaint.

A third admission to hospital "D" elicited several more tentative diagnoses, among them Addison's disease. During this period she had continued to consume 500,000 units of vitamin A per day and this "medication" was administered during her hospital stay as well. She was subsequently discharged from hospital "D" undiagnosed, with a rigid "poker" spine and an enlarged liver. Roentgenographic studies of the spine did not reveal any abnormality at that time.

Five months later she entered hospital "E" with multiple severe joint pains and marked difficulty in walking. X-ray studies showed "arthritic" changes of the spine and she was subsequently discharged with a diagnosis of infectious arthritis. For five years thereafter she was treated by chiropractors and osteopaths without improvement. She continued to consume 500,000 units of vitamin A faithfully each day.

Finally she went to a sixth hospital because of progression of the complaints for which she had been previously treated. At hospital "F," a relationship between her intake of vitamin A and her symptoms was suspected and the vitamin A content of her blood was determined. The fasting blood level of vitamin A proved to be 2000 micrograms per cent, the highest ever reported in man. Subsequently, a very careful work-up was done.

During the physical examination she complained of constant pain aggravated by any attempt to change her position. The area where a portion of her skull had been removed earlier was tense and bulged markedly. Dermatologic examination disclosed thinning of scalp and axillary hair, diffuse pigmentation of the skin and scaling of the palms and soles. Movements of the right shoulder and right hip were particularly painful and limited. Roentgenographic examination of the bones disclosed calcification in certain cartilagenous structures; however, the changes were not similar to those associated with infectious arthritis.

Immediately after the patient was admitted to the hospital all vitamin A medication was stopped and she was given a normal hospital diet. At the end of one month her skin texture had improved and her bone pains were markedly diminished. Two months after vitamin A medication had been stopped all spontaneous pain had gone and no further sedation was needed. Thereafter, she gained weight and improved steadily until all evidence of vitamin A poisoning had vanished.

In discussing the general problem of vitamin A toxicity, the authors emphasize the fact that vitamin A overdosage may produce intracranial disturbances which seem to be associated with an increase in the cerebrospinal fluid pressure. Apparently, either excess production or decreased absorption of spinal fluid results from vitamin A toxicity. The case presented by Gerber *et al.* is of particular interest, too, because she is the only adult yet reported to demonstrate bone changes. In this regard, the

fundamental disorder consisted of calcification with or without true bone formation of the pericapsular, ligamentous, tendinous and subperiosteal structures. The mechanisms responsible for these bone changes are obscure. It is known, however, that hypervitaminosis A can accelerate periosteal bone formation in experimental animals (S. B. Wolbach, *J. Bone Joint Surg.* **29,** *171 (1947)*).

Apparently the most common changes associated with excessive intake of vitamin A are skin lesions, of which coarsening of the skin (with itching) and thinning of the hair are most often reported. The cause of such skin changes is not understood; however, it seems significant that vitamin A deficiency produces somewhat similar skin lesions.

Since use of vitamin A is widespread, often indiscriminate and, indeed, occasionally intemperate, it is well to be reminded that chronic vitamin A poisoning in adults can be a very serious matter. That this case was a diagnostic problem for many years seems to underline the fact that nutrition is too often a neglected subject in the medical school curriculum.

THE RELATION OF DIET TO ATHEROSCLEROSIS

One of the outstanding problems in human nutrition is the relation of diet to the incidence of arteriosclerosis and degenerative cardiovascular disease. Many studies, both clinical and experimental, pertaining to this problem have been reviewed previously (*Nutrition Reviews* **8,** *176, 341 (1950)*; **9,** *167 (1951)*; **10,** *168 (1952)*; **11,** *72, 216, 231 (1953)*; **12,** *25 (1954)*).

A. Keys (*J. Mt. Sinai Hosp.* **20,** *118 (1953)*) has suggested that the fat rather than the cholesterol content of the diet may be of importance in determining the incidence of atherosclerosis. Statistics are reviewed which seem to show that the total age-specific death rate of adults is higher in the United States than in many other countries of the world. An increased incidence of "degenerative" heart disease, *i.e.*, myocardial infarction, chronic myocarditis and myocardial degeneration, was held responsible for this difference.

In support of the view that the level of dietary fat influences the total cholesterol content of the blood and the incidence of atherosclerosis, Keys has made studies of the blood cholesterol in individuals in European countries differing in dietary habits from this country. Forty-eight clinically healthy men were observed in Slough, England, eating diets having about the same fat content but one-half the cholesterol contained in the diet of age-matched groups of men in Minnesota. It was found that the total serum cholesterol of the two groups was nearly identical and showed a progressive rise from a mean of 178 mg. per cent at age 20 to 238 mg. per cent at age 50.

Quite a different finding was obtained in a study of 83 clinically healthy men, aged 20 to 56 years, living in Naples, Italy. Only 20 per cent of the calories was derived from fat in the diet eaten by these subjects. This is about one-half the dietary fat of United States males. Until the age of 30 the total blood cholesterol was not greatly different from that which was found in the Minnesota and British groups. At older ages significant differences occurred. In the Italian group the level of blood cholesterol actually fell between the ages of 40 and 50 whereas in men in Minnesota there was a mean rise of 29 mg. per cent during this age interval. Vital statistics from Italy are presented which indicate that the total death rate from age 40 to 60 is roughly equivalent to that occurring in the United States, but there were far fewer deaths from disease of the circulatory system in Italy. The available evidence indicates that among Italian males from 40 to 60 years of age the incidence of

degenerative heart disease was only one-quarter to one-fifth that occurring in the United States.

An additional correlated study of dietary habits, total blood cholesterol and the incidence of degenerative disease was made by A. Keys and co-workers (*Metabolism* **3,** *195* (*1954*)). Clinically healthy men and women below the age of 60 from Vallecas, a lower-class suburb of Madrid, Spain, were observed. Estimates were made of the dietary intake of these subjects. The calculated daily caloric intake per standard consumer unit was 2701. An additional group of 61 upper-class professional men were studied for comparison. Although dietary analysis was not carried out, it was assumed that the diet fat content was similar to that in the United States.

Anthropometric measurements demonstrated that the Vallecas men were shorter and thinner than the men in the professional class. This latter group approached the measurements found in the United States. The young women of the poorer classes were also found to be thin, but by the time middle age was reached they had reached prevalent weights for American women. The serum cholesterol values of the professional group were similar to those found in Minnesota and increased progressively with age. The serum cholesterol values of the Vallecas group corresponded to those found in Naples in that the younger individuals had values which were equal to those observed in Minnesota, but with age there was actually a decline rather than a rise. At the ages of 45 to 55 years the average serum cholesterol in the Vallecas group was much lower than in either the Spanish professional group or the Minnesotans. A rise in the average serum cholesterol in Vallecas women occurred until the age of 40 years with little change thereafter. Although there was a considerable difference in the relative fatness of the professional and Vallecas groups, this was eliminated by the authors as a full or partial explanation of the observed results.

Examination of the available vital statistics in Spain indicated that the deaths ascribed to all heart diseases in women were similar to those occurring in the United States. A striking difference was evident in the deaths from heart disease in male individuals in that the great augmentation in death rate from 40 to 60 years of age which occurs in American men was not evident in the Spanish men. Data on the incidence of heart disease in favored economic classes like the Spanish professional men studied were not available, though it was the opinion of local physicians that coronary heart disease is much commoner in this group than in the poorer classes.

The interpretation of these data presented by Keys and his associates may be hazardous. Much of the force of the argument depends on the accuracy of vital statistics. Granting the validity of the vital statistics, the possibility that variables other than the fat content of the diet, such as total caloric intake, are significant in the production of atherosclerosis cannot be excluded. Moreover, the difference in the means of the serum cholesterol values is small when compared to the wide variation of individual values. Despite these considerations the indictment of dietary fat deserves further careful study in view of the large and increasing proportion of fat in the American diet.

NUTRITION AND HUMAN GROWTH

The effects on the physique of school children of the siege of Jerusalem during the spring of 1948 have been described by H. V. Muhsam and A. Zaslany (*Statist. Bull. Israel* **1,** *No. 8* (*1952*)). The food supplies were inadequate for some five to six months,

from March to July/August 1948. During the entire siege, which lasted for about fifty days, no civilian supplies reached the city, including food, fuel, water and medical supplies. Actually, the food supply had been irregular since December 1947.

The data on height and weight of children are available for the beginning and end of the school year 1947/1948, *i.e.*, several months before and after the siege proper. The data do not indicate convincingly unfavorable effects on the increase in height. With the presiege data as the reference, boys of the same height weighed 2.1 kg. less, on the average, after the siege while for girls the mean difference was only 1.2 kg. Detailed dietary data were provided by M. J. Gutmann (*Acta Med. Orientalia* **10,** *49 (1951)*). In April the official rations provided 1360 calories per day, 960 in May, 670 in June, rising slowly and unsteadily in July until they reached about 2000 calories in August. In the adult population the weight losses amounted commonly to 5 to 8 kg., and often more, and were associated with physiologic manifestations of moderate degree of semi-starvation, including a reduction of the physical capacity for activity.

Extensive material on the growth of school children in Stuttgart, Germany, was assembled and analyzed by P. E. Howe and M. Schiller (*J. Applied Physiol.* **5,** *51 (1952)*). Records of weight and height, for some age groups, go back to 1910, and complete records for children 6 to 12 years of age are available since 1915. These data represent a unique record of variations in the average body size in a single community over a span of forty years. They are considered in reference to environmental factors which are complex, including shifts in economic conditions, housing, unrest due to wars and depressions, etc., but the changes in the availability of food are likely to be reflected most directly by the growth rate. There are no specific data on the economic status of the population, and detailed dietary information is limited largely to food available in Germany per capita per year in 1913, 1932 and 1938.

The results may be summarized briefly as follows: The data are surprisingly consistent in showing a secular trend between 1910 and 1940 toward increased height and weight in the numerous subgroups. Taking 10 to 11 year old folk school (Volkschule) boys as an example, we see that the average height was about 130 cm. in 1920, 136 cm. in 1930, and 137.5 cm. in 1940. The corresponding average weights were about 28.0, 29.0, and 29.5 kg., respectively. The long-term changes from year to year were greater in height than in weight and, in general, the height gains were most pronounced in the younger children. For the 18 to 19 year old high school (Oberschule) boys the over-all average increment was about 5 cm. in height and about 4 kg. in weight between 1920 and 1940. No suitable data for the final (maximal) height of adult men and women were available.

The data confirm the association of body size with the economic status of the parents, reported in previous studies. The Oberschule children, drawn chiefly from higher income families, were taller and heavier than the Volkschule children, drawn primarily from lower income families. In 1940 for 10 to 11 year old boys the means were 137.5 and 145.0 cm., and 29.5 and 34.5 kg., respectively.

Perhaps the most significant contribution of the study is the systematic documentation of the reduction of growth rates in both height and weight as the result of food shortages, whether due to governmental food rationing or to lowered purchasing power of the population. Marked decrements were seen during the period of the First, and, in particular, the Second World War. The unemployment crisis around 1930 is reflected only slightly in the growth curves. Keeping the age constant, the average difference in height between 1940 and 1945 was about 2 to 3 cm., with similar differences in weight (2 to 3 kg.).

The potential effect of vitamin B_{12} on the growth of school children was examined by N. Jolliffe, in collaboration with R. Funaro and a group of Italian workers (*Nutrition Symposium Series* **7,** *119 (1953)*). Two groups of children in the range of 6 to 12 years were followed for a period of seven months. One group lived in an orphanage while the other group attended elementary school in an economically depressed area. The diets were considered low in calories and, more importantly, in animal protein (17 to 27 g. per day), while the total protein intake was fairly satisfactory. In each locality the subjects were divided into two approximately similar groups, one receiving 20 micrograms of vitamin B_{12} daily by mouth, the other receiving placebo tablets, similar in appearance to the vitamin supplements. While the growth in height did not indicate significant differences between the control and the supplemented subjects, the average weight gains were higher in the treated subjects in 16 out of 19 comparisons of subgroups. While the individual comparisons frequently lack statistical significance, the pooled-treated and untreated subsamples showed a highly significant difference. In seven months the 172 children who received vitamin B_{12} made an average gain of 345 g. more than the group of 179 children who received the placebo tablets.

The authors do not go beyond a descriptive presentation of the results. Because of the ideal situation in the orphanage, it is unfortunate that the actual food consumption of the subgroups was not determined. This would have made possible the separation of the effect of the vitamin supplement on protein utilization and on increased food consumption, if any. In the study of N. S. Scrimshaw and M. A. Guzmán (*Nutrition Symposium Series* **7,** *101 (1953)*) there were some indications that the appetite of the children receiving the vitamin B_{12} (or aureomycin) supplements had increased. The authors sought to determine a factor in this increase which would help to explain the apparent positive growth response even though no food supplement was given.

The paper by Scrimshaw and Guzmán deals with several groups of subjects varying in dietary supplements and is not easily summarized. Nevertheless, it deserves careful attention. The subjects of the study were children 7 to 11 years of age in El Salvador and Guatemala, drawn from the lower economic levels of the population. These children were found to be two to four years behind United States children in height, weight and bone age. The diets were generally very low in animal protein (7 to 13 g.), vitamin A, riboflavin and calcium. Clinical examinations revealed no important signs usually associated with malnutrition, but the incidence of intestinal parasites was very high. Hematologic studies revealed no important deviations from normal values, except for low serum levels of vitamin A and carotene.

In the studies carried out in El Salvador no significant improvements in the rates of growth, as measured by height and weight, were obtained on providing a lunch rich in animal protein to children in one rural and one urban school. The same disappointing experience resulted when a lunch containing only vegetable protein was fed. The addition of 20 micrograms of vitamin B_{12} to this lunch had no effect on weight, but there was a tendency to an accelerated gain in height. It should be noted that this is in definite contrast to the data reported from Italy where weight but not height was positively affected by vitamin B_{12} supplementation.

In Guatemala, four comparable Indian communities were studied. The mid-morning snack provided animal or vegetable protein as well as supplementary calcium, vitamin A and riboflavin, and substantially improved the dietary situation with respect to all four nutrients. In two and one-half years on this dietary regimen there were some increases in the growth rate, but the individual variations were such that the mean differ-

ences could have been the chance result of sampling.

In two subgroups receiving vegetable protein and either vitamin B_{12} (20 micrograms) or aureomycin (50 micrograms daily), regarded as a substance "with animal protein factor activity," there appeared to be a slight increase in height gain and a more marked increase in weight gain. Neither of these differences reached the 5 per cent level of statistical significance.

In one village when only the vitamin B_{12} or aureomycin supplements were given without any additional food, increases in both height and weight over a period of eighteen months were greater than in the control group in another village. On suspension of all treatment for twelve months, the growth rate of the group receiving aureomycin initially dropped both in weight and height. In the "B_{12}" group, the cessation of supplementation was associated with decreased gain in height while the average gain in weight actually increased to the highest level observed in any group.

The rates of growth are importantly affected by diet. However, the difficulties involved in controlling the multiple environmental factors in human studies and in taking into account the genetic influences, together with such mundane elements as the length of time required and the expenses involved, are responsible for the fact that many important questions regarding the role of nutrition in human growth and development are still unanswered. The material presented in this review adds some important new data, but it does not provide conclusive quantitative evidence on the effect of diet in man's physical development and his final body dimensions.

VITAMIN K DEFICIENCY IN CHICKENS FED CORN-SOYBEAN DIETS

Considerable interest in vitamin K in practical poultry rations has been evident this past year. A "hemorrhagic syndrome" has been observed in commercial broiler flocks reared under field conditions and there is now some evidence that vitamin K deficiency may be involved. This is surprising in view of the earlier belief that ample vitamin K is present in rations for animals.

Several interesting studies in this connection have been published recently. Although these studies have been conducted under rather strict experimental conditions, the results obtained appear to shed light upon the syndrome seen in the field. It is important to point out that the rations used in each of these studies have been composed chiefly of corn (60 to 70 per cent) and soybean oil meal (30 to 35 per cent), long considered adequate in vitamin K. Also, the rations have been amply supplemented with necessary minerals and vitamins except vitamin K.

P. Griminger and co-workers (*Science* **118,** *379 (1953)*) used day-old crossbred chicks raised on wire screen floors. At the end of the experiment (four weeks) the chicks on the basal ration had an average clotting time of 9.8 minutes, whereas those given 5 mg. of vitamin K (menadione) per kilogram of diet had an average clotting time of 1.9 minutes, which is considered normal. Subcutaneous hemorrhages occurred in the legs and wings, and intramuscular hemorrhages in the thighs and breasts of an undisclosed number of chicks not getting the vitamin K supplement. Several drugs commonly used in commercial feeds for the prevention of coccidiosis or for their growth-promoting action were tested for their effect on vitamin K deficiency. The addition to each kilogram of diet of 100 mg. of para-aminophenylarsonic acid, 50 mg. of 3-nitro, 4-hydroxyphenylarsonic acid, or 15 mg. of terramycin in the absence of vitamin K significantly increased the clotting time to more than fifteen minutes. In all cases a

therapeutic dose of vitamin K brought the clotting time back to normal.

In similar and independent studies G. C. Anderson and co-workers (*Poultry Sci.* **33,** *120 (1954)*) also noted a hemorrhagic condition in chicks fed corn-soybean oil meal diets. These workers used New Hampshire chicks hatched from a normal flock fed adequate amounts of vitamin K. The chicks were raised on wire mesh floors for a period of six weeks. Again there was a marked delay in clotting time unless vitamin K was given prophylactically. Approximately 10 per cent of the animals died during the six-week period. Upon postmortem examination of the birds evidences of hemorrhage were most frequently found in the musculature of the breast and legs, in the meninges of the brain directly beneath the skull, and subcutaneously on the wings and legs. Pronounced gizzard ulcers were found in almost every bird examined.

Various supplements were tested for their effect on blood clotting time in the absence of supplemental vitamin K. Penicillin or sulfaquinoxaline seemed to have no effect on the deficiency at the levels tested, but the feeding of dehydrated alfalfa meal, fresh or dried feces, or vitamin K itself cured or prevented the condition. It is known that alfalfa and feces are good sources of vitamin K activity.

Further experiments on vitamin K have recently been reported by G. B. Sweet, G. L. Romoser, and G. F. Combs (*Poultry Sci.* **33,** *430 (1954)*). These authors used day-old New Hampshire chicks also raised on wire floors. The basal corn-soybean meal diet was similar to those used in the other two studies. After four weeks on experiment, chicks raised on the basal diet had a blood clotting time of eleven minutes, which the authors considered normal under their conditions and with the method used. The addition to this diet of 0.03 per cent of sulfaquinoxaline, or 0.03 per cent of para-aminophenylarsonic acid, significantly lengthened the clotting time to thirty-five and twenty-six minutes respectively. The addition of 0.02 per cent of terramycin was without effect. In other experiments when 1 to 5 per cent of dehydrated alfalfa meal or 0.36 mg. of vitamin K (menadione bisulfite) per pound of diet was added in addition to the mixture of drugs the clotting time was again normal.

It is obvious from these important studies that deficiencies of vitamin K may occur under certain conditions in otherwise normal chickens fed corn-soybean oil meal rations and raised on wire screen floors. Also, it is obvious that this deficiency may be markedly influenced by the presence of certain drugs used routinely in commercial poultry practices. The severe deficiency symptoms seen in these experiments are probably best explained by a combination of factors including the use of wire screen floors (thus preventing coprophagy), the use of solvent-extracted soybean oil meal rather than expeller meal, the use of low amounts of alfalfa meal or other sources of vitamin K, and the simultaneous use of certain drugs which may reduce intestinal synthesis of the vitamin. It is logical to assume that a real or "conditioned" deficiency of vitamin K is in part associated with the "hemorrhagic syndrome" seen under practical conditions among certain flocks of chickens fed rations similar to those used in the above laboratory studies. Vitamin K is now added to commercial poultry rations to a limited extent where this condition is seen. There are a number of unpublished reports indicating that vitamin K has some curative and preventive action, although this is a debated point and more experimental studies are necessary.

It is obvious that these studies may have an application to the nutrition of other animals and to man. Perhaps more attention should be given to vitamin K nutrition, especially in persons on special diets or those receiving drugs for one reason or another.

TRACE ELEMENTS IN PLANTS AND SOILS

During the last decade there has been a rapidly increasing interest in the trace elements. Probably the trace element most frequently referred to in the pages of this journal has been fluorine because of its striking relationship to the incidence of dental caries in human populations (*Nutrition Reviews* **5,** *322 (1947)*; **11,** *353 (1953)*). Possibly the next most frequently mentioned has been cobalt, in connection with the requirements of various ruminating herbivores for this element as well as its presence in the vitamin B_{12} molecule (*Ibid.* **11,** *114 (1953)*). Another trace element shown recently to have a definite relationship to animal nutrition has been molybdenum in its active role as a part of xanthine oxidase (*Ibid.* **11,** *250 (1953)*). During this period a most intensive series of studies has been conducted at the Department of Soils of the New Jersey Agricultural Experiment Station under the direction of F. E. Bear (*J. Agr. Food Chem.* **2,** *244 (1954)*). These studies have included various forms of laboratory, greenhouse and field work with boron, molybdenum, iron, manganese, zinc, copper, cobalt, iodine, fluorine and bromine. Various types of soil were examined for their total and available contents of these elements. Efforts were made to determine the optimum levels of these elements in crop plants and to diagnose deficiency and toxicity symptoms.

Boron deficiency symptoms included yellowing and reddening of tip leaves of alfalfa, cracked stems of celery, black areas in stem centers of cabbage, and various abnormalities in the twigs and leaves of trees. The standard remedy for boron deficiency was the application in fertilizer of from 10 to 25 pounds of borax per acre. For some crops as much as 50 pounds of borax were required, especially on overlimed heavy soils. The boron content of whole plants ranged from 5 to 100 parts per million (p.p.m.) based on dry weight. These values were readily increased by the addition of higher levels of borax to the soil.

The best known symptom of molybdenum deficiency in plants was in cauliflower where the older leaves became long and slender, consisting mostly of the midrib, and salable heads were not formed. In one field of 75 acres of New Jersey cauliflower, about 45 acres were unmarketable because of this deficiency. Alfalfa and tomatoes are other crops in which molybdenum deficiency has been noted. The suggested rate of application was not over 8 ounces of molybdic oxide or its equivalent of sodium molybdate per acre of acid soils, and half that quantity on soils with pH values above 6. The molybdenum content of New Jersey plants ranged between 0.3 and 5 p.p.m. on the dry weight of the material. These weights could be readily increased to as high as 100 p.p.m. by application of soluble forms of this element.

Iron deficiency was characterized by golden yellow foliage, especially on trees, and was observed to be widespread in irrigated arid regions. Increasing difficulty with iron deficiency has been experienced in fruit production, especially on coastal plain soils. The standard remedy has been to spray the trees with a 1 per cent solution of iron sulfate or to apply the salt under the spread of the branches at rates up to 30 pounds per tree. The iron content of New Jersey plants ranged normally between about 100 and 1000 p.p.m. of dry weight. Less than these amounts was commonly found in grains, tubers and other high carbohydrate parts of plants. Much higher values were occasionally found owing to precipitation on the leaves and accumulation within the plant or to contamination of the sample with soil.

Manganese deficiency symptoms were often similar to those of magnesium, except that manganese deficiency was noted on the

newer top leaves whereas that of magnesium occurs on the older lower leaves. The affected leaves had dark green veins with chlorotic tissue between. Leaves of small grains showed grey areas about half way between base and tip from which comes the name "grey speck disease." Crop yields often fell off before visible deficiency symptoms were noted. The manganese content of plants normally ranged between 10 and 100 p.p.m., but manganese values were higher when the soil pH value was permitted to drop below 5. Manganese deficiency was caused in some cases by overliming; this could be overcome by applying manganese sulfate at rates of 50 to 100 pounds per acre. It has also been successfully used in spray form at concentrations of 0.2 to 0.5 per cent.

Deficiencies of zinc occurred most frequently in trees and vines and were most common on highly calcareous and alkaline soils. The zinc content of plants and their component parts could be reduced by about one-half by raising the pH values of the culture solutions in which they were grown from 4.5 to 7.5. For example, oats had a zinc content of 19.5 p.p.m. when grown on soil with a pH value of 4.5. This was decreased to 5.9 p.p.m. when the pH value was raised by liming to 6.5. When zinc was observed to fall below 25 p.p.m., deficiency signs were commonly noted to develop in crop plants. The most spectacular form of zinc deficiency was "white bud" in corn, where the central bud and leaves turned white. The zinc content of plants normally ranged between 25 and 100 p.p.m. The standard remedy for zinc deficiency was to apply zinc sulfate in solid form to the soil under the branches of the trees or as a spray to the foliage.

The most common symptom of copper deficiency was a stunting and dying of the growth tips followed by initiation of growth from new points which also died. The only serious need for copper that has been demonstrated in the New Jersey area was in connection with crops grown on newly drained peat. The copper content of plants normally ranged between 2 and 20 p.p.m. of dry weight. Plants that have been sprayed or dusted with copper fungicides may show larger amounts of copper because of surface contamination. The standard treatment for copper-deficient soils was the use of copper sulfate applied at the rate of 50 pounds an acre once in five years.

No evidence of cobalt, iodine, or fluorine deficiency was reported for plant life. However, the cobalt content of hay was markedly reduced by heavy liming of the soil. It was also pointed out that cobalt sulfate is now being added to many thousands of tons of dairy cattle feed annually at the rate of about 2 g. for each ton of feed. No evidence of essentiality of bromine for plants was observed. None of the forage plants examined contained over 7 p.p.m. of bromine while tomatoes that were supplemented with potassium bromide accumulated as much as 2800 p.p.m. of bromine in the leaflets, 6300 p.p.m. in the stems, and 2100 p.p.m. in the roots without evidence of injury.

Some very surprising variations in trace element content of edible portions of 6 crops obtained from various areas in 18 states were found. For example, the iron content of tomatoes was found to vary from as low as 1 p.p.m. up to 1938 p.p.m. The manganese content of lettuce varied from 1 to 169 p.p.m. The molybdenum content of spinach was found to vary from undetectable amounts to 5.6 p.p.m. The cobalt content varied from undetectable amounts in cabbage, lettuce and snap beans to as high as 0.15, 0.19 and 0.26 p.p.m., respectively.

Some examples of the effective use of various types of fertilizers and particularly those containing trace elements in the reclamation of unusable tracts of land have been described by C. H. Kline (*J. Agr. Food Chem.* **2,** *404 (1954)*). The studies reported have largely been conducted in New Zealand and Australia where there are vast tracts of

almost completely useless land. One example was the coastal plain of Queensland which extends north from Brisbane for several hundred miles and inland from 10 to 30 miles. Though the rainfall was good, its sandy soils were so infertile that they have been considered too poor even to reforest. However, a recent study of the area indicated that the use of a fertilizer supplying phosphorus, potassium, calcium, copper, zinc, and molybdenum on the barren sand was capable of producing a vigorous clover yield.

In other areas in southern Australia, Tasmania and New Zealand, there were certain large tracts of pasture land of sufficiently rough contour to be impossible to cultivate. This pasture was capable of carrying only ⅓ to ½ sheep per acre. It was found that aerial distribution of clover seed and superphosphate into which a small amount of molybdenum had been incorporated could be conducted at a cost of approximately $5 per acre. This treatment was sufficient to improve the quality of plant life through the introduction of clover and also to increase the plant yields dramatically. The land became capable of carrying 2 and 3 sheep per acre, with a compensating increase in annual income of $12 to $20 per acre.

In other studies conducted at experiment stations in New Zealand, the use of as little as 2.5 ounces of sodium molybdate per acre on clover grass pasture fields improved the yield of hay from an acre from approximately 2800 to 4300 pounds. As much as 3 tons of limestone had to be used in order to obtain the same increase in hay production on parallel test plots. In some of these tests where the above amounts of sodium molybdate and lime were used simultaneously there was either no further increase in yield or a minor one. Though the first beneficial effect of molybdenum fertilization was not demonstrated until 1950, it was recognized to be such an important factor in the development and maintenance of good pasture land that within three years around one-half million acres or 1 per cent of the total land area of New Zealand was being fertilized in this manner. The comparative cost in New Zealand is approximately 25 cents per acre for the sodium molybdate as against $33 per acre for sufficient limestone to give equivalent yields.

Both of these series of experiments indicate conclusively the critical role that trace elements play directly in plant production, and ultimately in the support of animal life. Aside from the readily measured increases in plant production, there may well be a farther-reaching effect with respect to human welfare by reason of the increased amounts of trace elements incorporated into the plant tissues. Our knowledge about the requirements of the animal body for some of the various trace elements described in this review is very limited. It is well established that iron, manganese, zinc, copper, cobalt, molybdenum and iodine have indispensable roles in animal life and there is increasing evidence that fluorine in optimum amounts contributes to the development of caries-resistant teeth. To a varying degree for these several elements, we have a rather limited understanding about how much of each is required and how they contribute to the health and well-being of animals and man.

THE USE OF CARCASS ANALYSIS IN ESTIMATION OF AMINO ACID REQUIREMENTS

Protein is the most expensive dietary item for the human consumer and is also most likely to be a limiting nutritional component, particularly during growth and pregnancy. The requirement for protein is really a requirement for amino acids to be used in

protein synthesis and for replacement of endogenous losses. It is then obvious that the efficiency with which a dietary protein is utilized (referred to as its biologic value) will vary depending on the similarity between the amino acid composition of the dietary protein and the amino acid pattern required by the animal. These considerations point out the well known fact that proteins differ in their biologic values and in view of the importance and expense of protein in nutrition, methods of estimating their biologic value are of great practical importance.

The two most common methods for esti mating the biologic value of proteins are growth and nitrogen balance experiments. In the former, one determines the quantity of a protein required to produce maximum growth in animals receiving an otherwise complete diet or compares the growth-promoting effects of the protein under test with a standard of high biologic value such as whole egg protein. The nitrogen balance technic consists of measuring the retention of the amino acid nitrogen of the dietary protein by the growing animal or the ability of the protein to maintain nitrogen equilibrium in the adult. In the latter case one is measuring the ability of the protein to replace the endogenous losses of amino acids which occur throughout life. The nitrogen balance method has been used successfully in estimation of the amino acid requirements of the adult human being (*Nutrition Reviews* **8,** *118 (1950)*). In each of the above methods the data may be used to estimate the amino acid requirement of the animals. When the requirements for each of the essential amino acids are firmly established for a given species and for a given function it should be possible to estimate the biologic value of protein from an analysis of its constituent amino acids.

A third method for estimating amino acid requirement has also been employed. It is based on the assumption that the ratio of amino acids required by the animal will be similar to the ratio of amino acids present in the entire carcass of the animal. The experimental procedure consists of analysis of the entire carcass for its amino acid content. One then selects an amino acid the requirement for which has been established by other methods. This amino acid is assigned a value of 100 and the relative values of the other amino acids of the carcass are calculated. These relative values are then multiplied by the known requirement of the standard amino acid and their requirements are estimated.

There are some theoretic objections to this method. It has been well demonstrated that proteins in the body are in a dynamic state. Amino acids are withdrawn from the proteins and enter the general metabolic pool. A portion of these amino acids are deaminated and the nitrogen is excreted. These amino acids must be replaced from dietary sources. This phenomenon occurs throughout life and is responsible for the endogenous loss of nitrogen and the requirement for protein by an adult animal. It is known that various tissues of the body differ in their rate of protein turnover, that is, the rate at which the amino acids of the proteins are withdrawn and replaced. If these tissues have different patterns of amino acids the ratio of amino acids in the entire carcass would not give a true indication of the amino acid requirements. For these reasons it is desirable to compare amino acid requirements obtained by the carcass analysis method with values obtained by other methods. Such a comparison has been made by H. H. Williams and co-workers (*J. Biol. Chem.* **208,** *277 (1954)*).

These workers used rats, chicks and pigs in their experiments. They determined the amino acid composition of carcasses from newborn, weanling, and adult rats; 1-day-old and 10-week-old chicks; and from newborn, weanling, 33 kilogram and 93 kilogram pigs. Standard microbiologic procedures were employed for the amino acid determinations. The amino acid composition of the carcasses

was similar, there being no significant differences due to age or species. The values are given in detail and may be quite useful as reference material. Lysine was chosen as the standard amino acid and assigned a value of 100. The relative values of the amino acids obtained from carcass analysis were then compared to the reported requirements of these amino acids for growth of animals within each species studied. The latter values were taken from the literature and complete references are given. The growth requirements were obtained by growth and nitrogen balance experiments.

The data for rats indicate good agreement between the literature reports and the carcass analysis estimates for the requirements for 5 of the essential amino acids. Agreement was poor for arginine, histidine, methionine and tryptophan. In the case of arginine, for example, the value calculated from the carcass analysis was more than three times the value reported in the literature. There is evidence that rats are able to synthesize portions of their arginine and histidine requirements and this may partially explain the discrepancies. Both methionine and tryptophan have important functions in addition to their incorporation into proteins. Methionine is a methyl donor and a precursor of cystine while tryptophan is a precursor of niacin. These additional functions may contribute to the differences in the estimates by the different methods.

With chicks and pigs the amino acid requirements as estimated by carcass analysis agreed much better with the literature reports, in fact the agreement in most instances was quite remarkable.

These experiments indicate that the carcass analysis method is in general a good method for estimating amino acid requirements. One objection is that no distinction is made between the pattern of amino acids required for various functions such as growth, pregnancy, lactation, etc. When the exact amino acid requirements for all the various functions are known one should be able to assess accurately the biologic value of proteins by the relatively simple and inexpensive procedure of determination of their amino acid composition. This would allow very precise calculations of the supplementary effects of various proteins and should result in more efficient utilization of existing protein sources.

THE STRUCTURE AND PHYSIOLOGIC PROPERTIES OF ALDOSTERONE (ELECTROCORTIN)

Interest in the regulation of electrolyte metabolism by the adrenal cortex has been greatly stimulated by the identification on paper chromatograms of adrenal extracts of a substance twenty-five to one hundred times more potent than desoxycorticosterone (H. M. Grundy, S. A. Simpson, J. F. Tait, and M. Woodford, *Acta endocrinologica* **11,** *199 (1952)*; *Nutrition Reviews* **11,** *218 (1953)*). Progress has been rapid in this field since the above report. The isolation and crystallization of electrocortin was achieved by Simpson and her co-workers (*Experientia* **9,** *333 (1953)*). The same substance has been independently crystallized in this country by V. R. Mattox, H. L. Mason, A. Albert, and C. F. Code, *J. Am. Chem. Soc.* **75,** *4869 (1953)*.

A structure has been proposed for this compound on the basis of degradative studies by S. A. Simpson *et al.* (*Experientia* **10,** *132 (1954)*). The descriptive name of this compound is 11 beta, 21-dihydroxy, 3, 20 diketo-4-pregnen-18-al. This compound has the same chemical structure as corticosterone except that an aldehyde group is substituted for a methyl group on the eighteenth carbon atom. The definitive name of aldosterone has been proposed for this substance because of the unique presence of this aldehyde group.

In solution the compound exists largely as a hemiacetal formed between the 18 aldehyde and 11 hydroxy groups.

The small quantity of this substance thus far available has delayed a complete study of its physiologic properties. Preliminary reports have appeared from Basel of its action in rats (P. Desaulles, J. Tripod, and W. Schuler, *Schweiz. med. Wchnschr.* **83,** *1088 (1953)*) and in dogs (F. Gross and H. Gysel, *Acta endocrinologica* **15,** *199 (1954)*). The excretion of sodium, potassium and water during the two hours after the administration of desoxycorticosterone and aldosterone has been studied in adrenalectomized rats. A 50 per cent reduction in the excretion of sodium occurred on a dose of 0.16 micrograms of aldosterone whereas 4 micrograms of desoxycorticosterone were required for a comparable effect. The sodium-retaining power of both steroids was proportional to the logarithm of the dose. In promoting potassium excretion a dose of 6 micrograms of aldosterone per rat was required for a 50 per cent augmentation of excretion. A dose of 30 micrograms of desoxycorticosterone (DOC) was similarly effective. A difference therefore exists in the relative activity of these two steroids on sodium and potassium excretion in the urine. The ratio of the dose of aldosterone to desoxycorticosterone required for the same degree of sodium retention was 1:25. For promoting potassium excretion the ratio was 1:5.

A difference was also noted in the action of the two steroids on water excretion. In the acute test system used in these studies DOC was capable of decreasing water excretion by a mechanism which is probably independent of sodium retention. Aldosterone in doses ten times that required for near maximum sodium retention was without effect on water excretion.

Gross and Gysel have given aldosterone to adrenalectomized dogs. The dogs were maintained on desoxycorticosterone prior to aldosterone administration. The minimum dose of aldosterone capable of maintaining a compensated state was determined. This was found to be 1.5 to 2.0 micrograms per kilogram of body weight daily. Dividing the daily dose was more effective than giving it as a single injection. The hemoglobin rose during the switch from desoxycorticosterone (DOC) to aldosterone and it subsequently maintained this higher level. A slight rise in the concentration of nonprotein nitrogen of the blood also occurred. These changes took place even with doses of aldosterone which were considerably higher than those required to maintain life. The general health of the dogs receiving daily 1.5 to 2 micrograms per kilogram of body weight of aldosterone was as good as when they were receiving 50 to 60 micrograms per kilogram of DOC. In this test system therefore aldosterone was twenty-five to thirty times more potent than DOC.

A transient increase in the excretion of sodium, chloride and water occurred regularly on institution of aldosterone treatment. This was attributed to the disappearance of the hydremia (an increase in the fluid content of the blood) which develops in adrenalectomized dogs under DOC treatment. The plasma concentrations of sodium, potassium and chloride were maintained at normal levels during aldosterone administration. The ability to initiate a water diuresis after water loading was not improved by aldosterone. In this respect it acts in a way similar to DOC. Cortisone on the other hand will correct this defect in water metabolism of adrenalectomized animals.

The isolation, crystallization and establishment of the structure of aldosterone is an achievement of major importance. It is gratifying to witness the close international cooperation among three laboratories in Britain and Switzerland which has made these rapid advances possible. The initial limited physiologic studies indicate the great potency of the new steroid in restoring sodium excretion to normal. The promotion

of potassium excretion is relatively less potent than its effect on sodium retention as compared to DOC and it does not appear to have a direct antidiuretic action. It would appear that abnormal salt and water retention is less apt to occur with this steroid than with DOC, suggesting that its role in edema formation may be of relatively less importance than generally anticipated. The evidence thus far suggests that aldosterone is in fact the substance responsible for most of the adrenal action on electrolyte metabolism. Future research will have to determine the factors governing the release of this remarkable substance. Of interest to many will be the changes in aldosterone liberation in response to alterations in electrolyte intake in the diet.

QUANTITATIVE DETERMINATION AND PREPARATION OF THIAMINE AND ITS PHOSPHORIC ESTERS

None of the methods used hitherto allows the determination separately of thiamine and its phosphoric esters, monophosphorylated (MPT), diphosphorylated (DPT) and triphosphorylated thiamine (TPT) (*Nutrition Reviews* **4,** *148 (1946)*; **11,** *63 (1953)*). Chemical methods permit the separate determination on the one hand of thiamine and on the other of all its phosphoric esters together, either by utilizing the difference of the solubilities of the thiochromes resulting from the oxidation of thiamine and its phosphoric esters, or by determining thiochrome fluorescence before and after treatment with phosphatase which converts phosphate esters into free thiamine. Diphosphoric and triphosphoric esters can be determined manometrically, but free thiamine and the monophosphorylated ester are not reactive.

In a painstaking study D. Siliprandi and N. Siliprandi (*Biochim. et biophys. acta* **14,** *52 (1954)*) have elaborated a number of methods for separating thiamine and its esters from a mixture. The separation technics used were chromatographic (on filter paper, starch column and ion exchange units) and electrophoretic (using filter paper, starch column and cellulose powder columns). Paper chromatography was conducted using a number of different papers and solvents. After separation, the various thiochromes were formed and were detected by their fluorescence in ultraviolet light. A modification of the method permitted quantitative determination by eluting paper strips and "reading" the elution liquids in the Beckman spectrophotometer.

When a separation of thiamine esters in larger amounts was desired, a 100 cm. starch column was used. Four peaks were obtained when the extinction coefficients of the effluent fractions were determined spectrophotometrically. The first two peaks, corresponding to TPT and DPT, were completely separated from the last two peaks, containing MPT and thiamine respectively. The compounds in each pair of peaks, however, were not quantitatively separated but could be further identified by paper chromatography, and the compounds desalted by a series of adsorptions, elutions, and washings. Finally, chromatographic separation by ion exchange was conducted using a carboxylic acid exchanger and the "gradient elution technic," which consists of the use of a continuously changing eluant medium, produced outside the column in a mixing chamber. This method allowed the quantitative separation and recovery of all four components.

Electrophoretic procedures led to a similar degree of success. Paper electrophoresis, using a variety of buffers, separated the pure compounds without decomposition. A quantitative estimation was conducted by elution and ultraviolet reading of the eluates from the different electrophoretic bands. Starch column electrophoresis combined, in effect,

chromatographic and electrophoretic methods and gave good results. Even more satisfactory were cellulose powder columns which permitted an extremely clear-cut separation of thiamine and its esters. The purity of all separated fractions was tested by phosphorus determinations as well as by enzymatic decarboxylation assays.

The authors conclude this excellent report by a comparison of the suitability of the various methods in different situations and with various objects in mind. Paper chromatography and paper electrophoresis can be conveniently applied to the detection and determination of thiamine and its phosphoric esters in biologic materials. Both technics are useful for the entire separation of the esters in 10 to 100 microgram mixtures. For larger amounts, up to 2 mg., only paper electrophoresis can be used. The use of starch chromatography is limited to situations where only the separation between the enzymatically active components (DPT and TPT) and the inactive ones (MPT and thiamine) is required. Column electrophoresis, with cellulose powder as support, and ion exchange chromatography are suitable for the complete separation and determination of thiamine and its esters in a mixture.

The availability of satisfactory methods of separation and determination of thiamine and various thiamine phosphoric esters is fundamental to the detection and study of thiamine deficiency, still one of the most widespread nutritional diseases (*Nutrition Reviews* **9,** *141 (1951)*). Because of the fundamental role of thiamine-containing enzymes and the reported decrease of phosphorylated thiamine compounds in certain metabolic situations, diabetes in particular (*Ibid.* **10,** *19, 214, 349 (1952)*; **11,** *188 (1953)*), the application of the technics developed by Siliprandi and Siliprandi should prove fruitful.

ALTERNATIVE METABOLIC PATHWAYS FOR GLUCOSE METABOLISM

In recent reviews (*Nutrition Reviews* **12,** *154, 187 (1954)*) alternative pathways for fructose metabolism have been discussed. It is the purpose of the present review to discuss the divergent metabolic pathways by which glucose-6-phosphate may be broken down in animal tissues, *viz.*, the Embden-Meyerhof (glycolytic) and the hexose monophosphate (HMP) oxidative pathways.

The Embden-Meyerhof glycolytic scheme for the metabolism of glucose is now firmly established. It is well to recall that in this pathway as well as in the alternative HMP oxidative route the phosphorylation of glucose by adenosine triphosphate (ATP) catalyzed by glucokinase (hexokinase) is an obligatory first step and involves the expenditure of one mole of high-energy phosphate (approximately 12,000 calories) per mole of glucose. Glucose-6-phosphate, the product of this reaction in most tissues, may then follow an anabolic pathway and form glycogen via glucose-1-phosphate, or a catabolic pathway and form triosephosphate via the fructose-6-phosphate and fructose-1,6-diphosphate. In the latter case, another mole of high-energy phosphate is used. Under anaerobic conditions, the triosephosphates formed as a result of the fission of fructose-1,6-diphosphate are converted eventually to lactic acid. Under aerobic conditions pyruvate rather than lactate is the major end product of the Embden-Meyerhof scheme of reactions and this product is further catabolized via the Krebs tricarboxylic acid cycle to carbon dioxide and water. In the formation of pyruvate from triosephosphates two moles of high-energy phosphate are formed per mole of triosephosphate undergoing conversion.

That an alternative metabolic pathway to the Embden-Meyerhof route might exist

for glucose-6-phosphate was suggested by the work of O. Warburg, W. Christian, and A. Griese (*Biochem. Z.* **282,** *157 (1935)*) using red blood cells and Lebedew yeast extracts, and by F. Dickens (*Nature* **138,** *1057 (1936)*), also working with yeast enzymes. Warburg *et al.* were able to demonstrate oxidation of glucose-6-phosphate by red blood cells and yeast maceration extracts. They succeeded in preparing a glucose-6-phosphate dehydrogenase which they called "Zwischenferment." Triphosphopyridine nucleotide (TPN) was required as a co-factor, and the product formed was 6-phosphogluconic acid. Independently, Dickens was able to show the oxidation of glucose-6-phosphate and 6-phosphogluconic acid with separate yeast enzymes. Both Dickens and Warburg *et al.* found that pentose accumulated on further oxidation of 6-phosphogluconic acid. F. Lipmann (*Ibid.* **138,** *588 (1936)*) suggested that the pentose which accumulated might be arabinose formed by the decarboxylation of the intermediate, 2-ketophosphogluconic acid.

The above alternative oxidative route, variously referred to as the HMP oxidative, HMP shunt, or the Warburg-Lipmann-Dickens pathway, has been shown to be of importance in animal and bacterial metabolism by research in several laboratories. F. Dickens and G. E. Glock (*Biochem. J.* **50,** *81 (1952)*) have obtained direct evidence of the TPN-dependent oxidation of glucose-6-phosphate, 6-phosphogluconate and pentose-5-phosphate by enzymatic extracts from liver, kidney and brain of rat and rabbit. Their data, furthermore, favored the hypothesis that 6-phosphogluconolactone was the first intermediate in the oxidation of glucose-6-phosphate. This mechanism, which visualizes the reaction as a simple dehydrogenation of glucose-6-phosphate in its commonly occurring pyranose ring form, has been confirmed by O. Cori and F. Lipmann (*J. Biol. Chem.* **194,** *417 (1952)*).

Additional elucidation of the mechanisms of pentose formation in the HMP oxidative pathway has been accomplished by B. L. Horecker and co-workers. Using a purified yeast preparation, Horecker, P. Z. Smyrniotis, and J. E. Seegmiller (*J. Biol. Chem.* **193,** *383 (1951)*) demonstrated the formation of two pentose phosphates during the oxidation of 6-phosphogluconate. They were identified as ribulose-5-phosphate and ribose-5-phosphate. The data of these investigators suggested that 6-phosphogluconate was first oxidized at carbon 3 to form 3-keto-6-phosphogluconic acid which spontaneously lost carbon dioxide to form the pentose, ribulose-5-phosphate. Through the agency of a pentose phosphate isomerase in these preparations the latter compound was converted to ribose-5-phosphate to the extent of 70 to 80 per cent.

A similar conversion of 6-phosphogluconate to pentose phosphate was obtained with enzyme preparations from rabbit liver and bone marrow by J. E. Seegmiller and B. L. Horecker (*J. Biol. Chem.* **194,** *261 (1952)*). Horecker and P. Z. Smyrniotis (*Ibid.* **196,** *135 (1953)*) demonstrated the reversibility of the oxidation of 6-phosphogluconate by obtaining a net synthesis of this compound from ribulose-5-phosphate and carbon dioxide.

E. Racker (*J. Biol. Chem.* **196,** *347 (1952)*) and others have shown that ribose-5-phosphate is further metabolized to glyceraldehyde-3-phosphate (triosephosphate) and a C_2-fragment not definitely identified as yet. (According to F. Dickens ("Hormonal Factors in Carbohydrate Metabolism," *Ciba Foundation Colloquia on Endocrinology* **6,** *pp. 12–13 (1953)*), it is generally agreed that free glycol aldehyde does not appear.) It is thus seen that at the point of formation of triosephosphate, the HMP shunt rejoins the Embden-Meyerhof scheme and further oxidative catabolism of the triosephosphate proceeds via pyruvate to carbon dioxide and water.

It has been evident for some time that there is a wide gradient in the over-all rates

of oxidation of glucose in different tissues as determined *in vitro* by incubation of slices with C^{14}-evenly labeled glucose. R. E. Olson, J. S. Robson, H. Richards, and E. G. Hirsch (*Fed. Proc.* **9,** *211 (1950)*) demonstrated that whereas slices of rat brain, kidney and heart tissue produced sizeable amounts of $C^{14}O_2$ from the tagged glucose, liver slices were relatively inactive. Even tumor tissue showed a greater rate of glucose oxidation to carbon dioxide than did liver slices (Olson, *Cancer Research* **11,** *571 (1951)*; P. C. Zamecnik, R. B. Loftfield, M. L. Stephenson, and J. M. Steele, *Ibid.* **11,** *592 (1951)*).

Experiments to determine the extent to which each of the above pathways contributes to glucose catabolism in different tissues were recently undertaken by B. Bloom and DeW. Stetten (*J. Am. Chem. Soc.* **75,** *5446 (1953)*) and by Bloom, M. R. Stetten, and DeW. Stetten (*J. Biol. Chem.* **204,** *681 (1953)*). These workers measured the amount of $C^{14}O_2$ formed from uniformly labeled and C-1-labeled glucose and from C-1, C-2, and C-3-labeled lactate, both *in vivo* and *in vitro*, and calculated, on the basis of certain reasonable assumptions, the relative contributions of the Embden-Meyerhof glycolytic and the HMP nonglycolytic pathways of glucose catabolism in the whole animal and in individual tissues. They found that in the intact animal and in isolated diaphragm slices the pathway was almost exclusively glycolytic whereas in kidney and more especially in liver there was evidence for high activity of the nonglycolytic HMP shunt. In liver slices less than 25 per cent of the glucose oxidized was catabolized via the Embden-Meyerhof scheme. In attempting to reconcile the data in the intact animal with that obtained from liver and kidney slices, Bloom *et al.* considered that either (1) the glucose catabolized by striated muscle via glycolysis with intact animal overshadows the contribution made by liver and kidney, or that (2) the preponderance of the nonglycolytic pathway in liver slices may be an artifact of the experimental conditions.

Although K. F. Lewis, H. J. Blumenthal, C. W. Wenner, and S. Weinhouse (*Fed. Proc.* **13,** *252 (1954)*) have confirmed the finding of a high degree of nonglycolytic catabolism of glucose in liver slices, some doubt still remains concerning the extent of this pathway. J. Katz, S. Abraham, R. Hill, and I. L. Chaikoff (*J. Am. Chem. Soc.* **76,** *2277 (1954)*) have shown that the derivation of the equations by Bloom *et al.* was questionable. Applying the data of Bloom *et al.* to revised equations, Katz and his co-workers showed that at least 80 per cent of the carbon dioxide derived from glucose in the liver could have arisen via glycolysis.

Olson (*Cancer Research, loc. cit.*) has presented data indicating that phosphohexokinase, the enzyme phosphorylating fructose-6-phosphate, is very low in normal liver and may be the limiting step in glycolysis in that tissue. The low concentration of this enzyme might necessitate the channeling of some glucose catabolism through an alternative system.

There seems little doubt that biochemical systems constituting alternate pathways for glucose catabolism exist in animal tissues and that the degree to which each controls glucose metabolism in a given tissue is a function of the basic enzyme profile and probably also of hormonal control. Pathways, in addition to those mentioned, such as the direct oxidation of glucose (D. C. Harrison, *Biochem. J.* **25,** *1016 (1931)*; M. Nakamura, *J. Biochem. (Japan)* **41,** *67 (1954)*), may also exist for glucose in animal tissues.

This recent reappraisal of alternate pathways for glucose is most stimulating and opens the way not only for further study in this area, but also should prompt new researches in other areas of metabolism for alternate pathways that underlie the amazing biochemical flexibility of protoplasm.

IRRADIATION EFFECTS ON LINOLEATE OXIDATION

Ionizing radiations have the property of inducing a chain reaction which in many respects is similar to autoxidation. Such a reaction was reported by J. F. Mead (*Science* **115,** *470 (1952)*) to occur when linoleic acid was exposed to irradiation. Since linoleic acid is one of the unsaturated fatty acids essential in nutrition and is universally present in animal tissues, this observation provided a clue that irradiation damage might represent a nutritional disorder.

Furthermore, this observation created interest in the mechanism involved in the preservation of foods by ionizing radiations and also prompted investigations regarding the possible effects of naturally occurring antioxidants in foods on the radiation effects. A recent study by B. H. Polister and J. F. Mead (*J. Agr. Food Chem.* **2,** *199 (1954)*) reports on the influences of vitamin A, tocopherol, calciferol, ascorbic acid, cysteine, glutathione, and a commercial antioxidant (2,6 di-*tert*-butyl-4-methylphenol) on the autoxidation of the methyl ester of linoleic acid (methyl linoleate) as induced by ionizing radiations.

The effect of these antioxidants was studied with the methyl linolate dispersed in a system containing Tween 80 and water to form an oil-in-water emulsion with particle size of about 5 microns. Both irradiated and unirradiated emulsions were treated with 95 per cent ethyl alcohol and the solutions thus formed were analyzed spectrophotometrically for oxidation products of methyl linoleate. Varying concentrations of the antioxidants as additions to the emulsions were tried and their influences on autoxidation of linoleate as well as their own destruction were determined.

Vitamin A at low concentrations was almost completely destroyed by ionizing radiations in the presence of linoleate. At high concentrations it was effective in protecting the linoleate against oxidation. Calciferol afforded partial protection for linoleate and was itself stable to the radiations. Both the commercial antioxidant and tocopherol prevented the oxidation of linoleate. These were by far the most effective antioxidants studied. Gamma-tocopherol was completely protective at all concentrations above 2.69×10^{-5} molar (0.0016 per cent).

Ascorbic acid was completely protective against linoleate oxidation at high concentration, and was moderately protective at low concentrations. At all concentrations tested (0 to 2.65×10^{-3} molar), the vitamin itself was partially destroyed by ionizing radiations.

Both cysteine and glutathione afforded some protection against linoleate oxidation by ionizing radiations, but the concentrations required for protection were much greater than was the case with tocopherol or the commercial antioxidant. Partial destruction of the cystine and glutathione also occurred as a result of irradiation.

As was expected, the lipid-soluble antioxidants were much more effective than the water-soluble forms in protecting linoleate from oxidation by ionizing radiation. Furthermore, the potent lipid-soluble antioxidants were themselves much more stable to irradiation than the nonlipid-soluble forms.

The possible role of tocopherols in protecting against ionizing radiations in man or animals is a question that would be raised as a result of the studies of Polister and Mead (*loc. cit.*). The lowest concentration of tocopherol that protected linoleate in their *in vitro* studies is within the range of tocopherol concentration in many animal tissues and in blood plasma.

NOTES

Carotene Destruction During Haymaking

The rapid destruction of carotene in alfalfa during field curing has been attributed to losses by both enzymatic and photochemical processes. Continued variation of temperature, moisture, light intensity, and physical conditions within the plant tissues all tend to make the separate evaluation of these processes very difficult. K. A. Walsh and S. M. Hauge (*J. Agr. Food Chem.* **1,** *1001 (1953)*) attempted to evaluate these factors by studies on incubated aqueous suspensions of macerated alfalfa leaves under controlled conditions.

The enzyme system responsible for carotene destruction had a temperature optimum of 43°C., was heat-labile, partially inhibited by cyanide, and was more active at pH 4 to 5 than at higher pH values. Photochemical destruction of carotene was insensitive to pH changes between 4 and 8, to temperature changes between 10° and 45° C., and to prolonged heat treatments after enzyme inactivation. Additional loss of carotene in the absence of enzymatic or photochemical destruction was attributed to autoxidation, which is of minor importance below 40° C. and is little affected by pH changes. Both destructive processes are important in field curing and both must be controlled if carotene losses are to be decreased.

Antibiotics and Nitrogen Utilization by Cattle

Increase of growth rates of swine fed certain antibiotics has stimulated interest in the effects of antibiotics on utilization of nonprotein nitrogen, such as urea and ammonium salts, by cattle. J. M. Prescott (*J. Agr. Food Chem.* **1,** *894 (1953)*) removed the rumen contents from freshly killed steers, removed protozoa and vegetable matter, and then studied the activities of the remaining bacteria. Glucose and urea were added to the cultures, along with various levels of antibiotics. After eight hours incubation at 37° C. combined urea plus ammonia nitrogen was determined.

Steers fed on fresh pasture and green roughages could utilize these forms of nitrogen far better than those fed largely on grain, by a ratio of 10:1. This is a reflection of differences in the microflora developed by use of the two feed materials. Four antibiotics were used: aureomycin hydrochloride, terramycin hydrochloride, bacitracin, and L-ephenamine penicillin. When these were added to the rumen contents of steers fed on a grass-roughage diet, reduced utilization of nonprotein nitrogen resulted in every case. Concentrations of antibiotics varied from 0.5 to 250 micrograms per milliliter of rumen liquid. These results indicate the desirability of *in vivo* experiments on cattle fed a diet rich in nonprotein nitrogen. Use by cattle of cheaper sources of nitrogen would have significant effects on the cost of meat and milk production.

The 1954 Babcock-Hart Award

The Babcock-Hart Award in recognition of outstanding accomplishments in the fields of food research and technology was presented June 29 to Dr. Edwin John Cameron, Director of the Washington Research Laboratories of the National Canners' Association during the annual meeting of the Institute of Food Technologists in Los Angeles.

This annual award is made possible by a grant provided by The Nutrition Foundation, Inc. Dr. Cameron was cited for his contributions in technologic improvement in the nutritive quality of foods.

Nutritive Value of Canned Food

Since 1942 many data have been accumulated on the nutrient contents of various canned foods. In 1950 a review of these data indicated a lack of balance with regard to the number of analyses available for the vitamin content of some products. The need for further work was obvious because of advances in analytic methods and in determining the significance of newer vitamin factors in human nutrition. Wisconsin workers, L. J. Teply, P. H. Derse, C. H. Krieger, and C. A. Elvehjem (*J. Agr. Food Chem.* **1,** *1204 (1953)*), have therefore extended previous studies in this field to fill the informational gaps. Sixteen new products were introduced into the study.

Samples were shipped under code to the Wisconsin Alumni Research Foundation for assay. Composite samples of the edible portions were prepared and accepted methods of determination were employed. Forty-eight canned products were examined. The human requirements for vitamin B_6 and folacin are considered to be of the order of 1.5 mg. of vitamin B_6 and 0.1 to 0.2 mg. of folacin per day. Servings of 100 g. of many products studied would make substantial contributions toward one day's requirement of these two vitamins.

The highest levels of vitamin B_6 were found in corn, cherries, pineapple juice, potatoes, sauerkraut, tomatoes and meat products. The products highest in folacin were asparagus, beans, berries, boned chicken, peas, pears, spinach and oysters. Results on other vitamins are in good general agreement with earlier results except that the present figure for beta-carotene in sweet potatoes is 50 per cent higher, that for thiamine in boned chicken is higher but for tuna considerably lower, values for riboflavin are 30 per cent lower, and most of the niacin results are somewhat higher. No ready explanation for these differences is available, but in both studies considerable variation was found in these analyses among various samples.

The Effect of Freezing on Pork Quality

In view of the widespread use of home freezers, information concerning the effects of freezing on the quality of meat is of considerable importance. F. A. Lee and coworkers (*J. Am. Dietet. Assn.* **30,** *351 (1954)*) have reported the influence of freezing at three different temperatures on the vitamin content and palatability of pork. Pork chops were frozen at −46°C. (equivalent to commercial quick-freezing), −17.8°C. (equivalent to home deep freezing), and at 0°F. Samples were taken for analysis before freezing, immediately after freezing, and after six months of storage. Thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, and total solids were determined in raw and cooked samples. Also, palatability was estimated by arbitrary scoring of the cooked product by judges. It was found that neither palatability nor vitamin content was affected by any of the freezing or storage conditions studied. It seems quite probable from these results that the nutritional value of frozen meat is equivalent to that of the fresh product.

Rowett Research Papers

The Rowett Research Institute, Bucksburn, Aberdeenshire, Scotland, has published the annual list of their research papers for the academic year 1952–1953, together with brief summaries. A short history of the Institute, as well as a list of its staff, is included. Copies may be obtained from the Librarian at a cost of 2/6d, postage free.

Recent Nutrition Books

Biochemical Preparations. Vol. III. Edited by E. E. Snell. John Wiley and Sons, Inc., New York, N. Y., 1953. Pp. 128. Price: $3.50.

Map—*Study in Human Starvation Diets and Deficiency Diseases.* Prepared and published by the American Geographical Society, Broadway at 156th Street, New York 32, N. Y., July 1953. Price: $1.25 folded; $1.50 flat (in roll).